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CONTRIBUTIONS TO THE CYTOLOGY OF SOME OF THE CUCURBITACEAE.

PLANTS CONSIDERED.

1. *Momordica charantia*.
2. *Trichosanthes dioica*.

INTRODUCTION.

Cucurbitaceae is a large dicotyledonous family and is widely distributed, with its 400 species in warmer parts of the whole globe, especially in the tropic (Hooker 13). It is rare in the colder regions. Only three genera occur in Europe. Ecballium is monotypic and is confined to the Mediterranean region, where Citrullus colocynthis also occurs. Bryonia dioica is the sole British representative spread from the Mediterranean region into South and Central Europe.

Regarding its systematic position, the older Botanists such as Robert Brown, De Candolle and Naudin placed it with Passifloraceae among the perigynous and polypetalous families and this view was also adopted by Bentham & Hooker (1). Eichler on the other hand, placed it near Campanulaceae as an appendage to his series Campanulinae basing his decision on the typically epigynous pentamerous flowers, but frequently gamopetalous corolla, the tendency to union of stamens and the form of calyx with narrow yet plainly leaf-like points. This view has been followed by Engler (10) who places the family by itself in order Cucurbitales (J. Hutchinson (14), Hooker (13), Collet (5)) next to the order campanulales. There are however strong reasons for adopting the older view. The important characters of the ovule structure which has a large persistent nucellus frequently an extensive tapetal tissue, and two distinct integuments, are at variance with a position among the typical sympetalous families, but find a parallel in Passifloraceae and allied families, in which there is also a tendency to union of the petals and epigyny is noted. Rendle (24) therefore has

given it a position suggested by Hallier to regard it with Begoniaceae (and the small family Datisceae) as forming a distinct order next to Passifloraceae group of families.

Recently P. Vuillemin (Annales d. Sciences Naturelles, Botan ser. X, V, 5(1923)) has mentioned that cucurbitaceae and Begoniaceae must be relegated to the apetalous group of orders, among which he classes them with Belanophoraceae, Rafflesiaceae, Datisceae, Nepenthaceae and Aristolochiaceae. He regards them as apetalous and the so-called corolla as an inner calyx.

There are both wild and cultivated species, a lot of work has been done in Europe on the plants occurring there ~~being~~ by different workers, prominent ^{among} ~~them~~ being L.F. Heimlich(11), Edward F. Castetter(3), Sara F. Passmore (22), whose work exist in English literature and Kozhuchows, Kirkwood, Strasberger, Boenickes' work is found in German papers. Little or no work seems to have been done on megasporogenesis. There has been a tendency in our laboratories to work on Economic plants. Cytological work has been done on Brassica oleoracia of the family Cruciferae. Mr. P.C. Joshi is also carrying on his research on some Economic plants. It was suggested therefore to the writer by Dr. S.L. Ghose who thought it desirable to carry on research on Momordica charantia(being an economic plant) and for comparison sake on Trichsanthes dioica which is both wild and cultivated, being distributed throughout the plains of North India extending to Assam and Eastern Bengal.

MATERIAL & METHOD.

The flowers of all ages from Botanic Gardens were fixed in November 1935, between 12 to 2 p.m. The division time by ordinarily teasing the anther on a slide and staining it with acetic methyl blue was ascertained. Fixation was done in many fixatives but best results were given by Allen's modification of Bouin's fluid (McClung 20).

Formula:-

- A. Picric acid (Saturated solution) aqueous 75 cc.
- Formol 25 cc.
- Glacial acetic acid 5 cc.
- Urea 2 grms.

- B. 0.5 gm. of chromic acid to be added to every 35 cc. of A. before fixation.

In case of buds which were covered by minute hairs the petals and sepals were carefully removed before fixation. For fixing flowers for mitoses stages, the exhaust pump was used for immediate fixation.

The material thus fixed was kept in the fixative for 24 to 30 hours after which it was washed in water for 5 to 10 minutes and immediately transferred to 2½ % alcohol. Then the material was passed through 5%, 7½%50% alcohol keeping in each grade for about 4 hours. Finally the material was kept in 60% alcohol. After complete dehydration of the material in 70% absolute alcohol(changing last grade twice) the usual paraffin method given in Chamberlain(4) was followed.

The sections were cut 8-12 μ thick. Great difficulties were experienced in examining the slides of megasporogenesis because of the fact that each nucellus is about 40 to 60 μ thick and is cut in 4-6 sections. The early stages are cut in 1 or 2 sections.

Haidenhains iron alum haematoxylin was mostly used for the staining of sections. Besides destaining with 2% iron alum, a concentrated solution of Picric acid was also used (Tuan 29) which also gave quite satisfactory results. ~~L.R. Taylor's~~ Iron acetocarmine method of fixing and staining was also tried on fresh buds, but no satisfactory results were obtained.

Drawings were all drawn with the camera lucida and in some of the cases positions of chromosomes were located ordinarily, because the light under oil immersion lenses and the camera lucida becomes very faint and the exact position of the chromosomes could not be traced. In cases of the Embryo^{sac} formation the writer has tried his utmost to draw the nuclei in their real positions, because they occur in as many as 4 to 6 sections. The nucellus of the family being very large and persistent.

The megasporogenesis of Trichosanthes dioica could not be successfully carried out due to scarcity of material.

MOMORDICA CHARANTIA.

Flowers of Momordia charantia are monoecious, bract about the middle of the male pedemncle, obmucular, entire, calyx lobe ovate, acute; petals $3/8$ to $3/4$ inch, yellow, five, free. Stamens inserted at the base of the calyx, five in number and free. Ovary of the flower^e is inferior, 3 carpellary, style is one with 3 stigmas, placentas are also 3, vertical in double lines, the edges of the carpellary leaves are turned in so far that the ovary(in flower time even is spuriously 3-celled. Ovules are many, horizontal and are cut therefore longitudinally in cross sections only.

DEVELOPMENT.

Previous work:- A search through the available literature and reference works indicate that only microsporogenesis of the family has attracted a lot of attention, but very little information is available regarding megasporogenesis. Most of the earlier workers have only been tracing the life history and have not entered into deep study of cytology. Mr. L.F. Heimlich(11) only studied the development of microspores of Cucumber, giving no reference to the nature and structure of microspores at all. He seems to have only dealt with the outlines of his work. Edward F. Castetter(3) has worked on microsporogenesis of Cucurbita maxima and has criticised the strong tendency which has existed for may decades among

investigators to regard the division of the pollen mother cells in higher plants by a process of cell plate formation: Farr writes that in g higher plants the pollen mother cells in forming the microspores usually undergoes a process of furrowing and that no cell plate is formed or at best only sporadic attempts occur to form cell plates. Castetter(3) has also supported the views of Farr. He has gone deeply into the study of Cytology and reports a multipolar spindle, he writes "just previous to diakenesis the cytoplasm immediately surrounding the nucleus becomes densely granular forming a rather compact region around the nucleus, this region has been referred to by several workers as peri-nuclear zone. As the nuclear membrane disappears numerous beaded fibres of nuclear origin appear in the nuclear cavity. These fibres form a web in which the chromosomes are seen. The fibrous web soon projects out into three or more cones constituting the multipolar spindle, which gradually becomes arranged in two groups to form the bipolar spindle. A somewhat similar method of origin of multipolar spindle has been described in Larix by Allen and recently by Santos in Elodea." He refers again to the appearance of distinct cell plates at the equator of homotypic spindles and says "such plates do appear in the heterotypic spindle but never extend beyond the limit of the spindle and thus never reach the plasma membrane. These heterotypic and homotypic spindles leave on their paths at the time of disappearance a band of densely granular material which is doubtless a product of the disintegration of the spindles which soon become dissipated. Quadripartition of pollen mother cells^{is} effected by partition walls which originate on the inner surface of the special wall formed by the

secretion of the cytoplasm just surrounding the pollen mother cells. These partition walls at first grow centripetally by the deposition of callose on their inner edges. Thickenings appear on the strands of the cytoplasm midway between each pair of nuclei. These thickenings condense, fuse side by side and then become incorporated into the ingrowing septum. The septa unite at the centre of the cell dividing the mother cell into four uninucleate protoplasmic masses, the microspores.

Then in 1930 Sara F. Passmore(22) worked upon many plants belonging to this family and summarises her work as ~~follows~~ follows:-

A. Darkly staining granules are present among the bivalent chromosomes in late diakinesis in all members of Cucurbita, but are absent in Citrullus, Luffa and Cucumis.

B. She also reports the presence of special wall which grows centripetally to divide the pollen mother cells into 4 pollen grains.

MOMORDICA CHARANTIA.

The Anther.

Microsporogenesis:- The young stamens is a sort of protuberance from the base of the petals. The anther is at first a homogenous mass of small meristematic cells(Fig.1) covered over by an epidermis. The cells all are nearly alike and there seems no tendency in the cell for the formation of the archesporial tissue. The cells are densely protoplasmic and the outline of the anther is more or less oval in the cross section, with continued growth it becomes many lobed, the lobes increasing as the flower grows. In earlier stages it becomes bilobed, then trilobed, and remains trilobed even in very old flowers. The primary archesporium is differentiated almost simultaneously with the appearance of the two lobes of the anther ~~of~~ which consists of 2 to 3 cells in cross sections. ^(Fig 2+3) ↑ The archesporium in this case is deep seated, but a hypodermal archesporium is reported by L.F. Heimlich(11) in Cucumber. The archesporium cells show the usual features which always distinguish them from other cells of the anther. They are large in size, possess more densely staining cytoplasm and larger nuclei. The anther lobes go on becoming more and more prominent after the differentiation of the primary archesporium. These archesporium cells cut off parietal tissue on the outside by the further division of which both ~~par~~^{par}iclinally and anticlinally two to three layers of parietal tissue are formed, which intervene between the epidermis and the sporogenesis cells of the anther(Fig. 3). Outermost parietal layer becomes later on differentiated into an endothecium and the

innermost layer into a tapetum which remains as a jacket of nourishing cells in immediate contact with the sporogenous tissue. The tapetum has no definite morphological boundary or origin but results from pressing into special physiological service the sterile cells of whatever origin contiguous to the sporogenous tissue. (Coulter & Chamberlain 7). The sporogenous cells take definitely no part in the formation of the latter. The nature of the tapetum is described later on.

The division of the sporogenous cells occurs and as a result of this 3 to 12 most commonly (4-5) microspore mother cells are seen in a cross section of an anther lobe (Fig. 7a), the number decreasing towards the two extremities. All the cells do not divide at the same time nor do they divide in the same plane. On the whole nearly 40 microspore mother cells have been counted in a longitudinal section. ^{Fig 6} As each microspore cell gives rise to 4 pollen grains the total output number of the pollen lobe of each stamen in Momordica charantia varies from $40 \times 4 \times 4 = 640$ and $40 \times 5 \times 4 = 800$.

The tapetal cells show the usual characters, oblong shape in section, broad, large nuclei, richly protoplasmic contents, and a capacity to stain as deeply or even more deeply than the microsporocytes. The complete organization of the tapetal jacket is coincident with the mother cell stage but the greatest development of it is during the formation of the tetrad. They have in that stage enlarged very much. The cells remain uninuclear throughout in Momordica charantia excepting in very few cells, although it is very common for the enlarged tapetal cells filled

with food material to become binucleate or even tri-nucleate and multi-nucleate in other plants (Coulter & Chamberlain 7). These tapetal cells have distinct vacuoles also present in them. These cells always remain at the periphery when their inner walls become disorganised and never push themselves in between the pollen grains to form the periplasmodium.

The mother cells undergo a period of rest, their nuclei increase very much in size. Unlike flowering plants in general, the mother cells do not round off and separate from each other, nor do their walls become thickened; but remain packed together as is the case in Digera, Althaea etc. The walls of the tapetum remain persistent but the cytoplasm always contracts towards the peripheral walls, as in (Fig 7b). The cytoplasm of the spore mother cell may become rounded and float in the cavity of the cell but the entire cells do not become rounded. It may be due to as explained by Chamberlain & Coulter (7) as such " This may be due to limitation of space due to tardy disorganization of tapetum or its failure to disorganise", this explanation particularly the latter part of it holds good here. Thus the spore mother cells just before entering the first or the heterotypic reduction division are polygonal in outline and the cell walls are thin. Under the walls are found definite layers which again seem to be secreted from the cytoplasm forming a sort of thick wall between the actual wall and the floating mass of cytoplasm of the spore mother cell. Cytoplasm is alveolar in structure and contain definite deeply staining granules. Sara F. Passmore (22) has worked upon many members of

Cucurbitaceae but has not described anywhere the occurrence of these darkly staining granules. On the contrary she has reported darkly staining bodies among the pairs of chromosomes which lie scattered through the nucleus.

Each cell has one spherical nucleus in the centre with a fine nuclear membrane. Generally at this stage ⁱⁿ the nucleus is reported a nucleolus which stains deeply with haematoxylin in many other plants, of this family as well as of many other families; but in the case of Momordica charantia no such case has been found out.

The heterotypic division begins with an increase in the density of the karyoplasm and closely following this the linin threads make their appearance. Numerous minute and faintly staining granules become distinct on the linin and it resolves into the usual reticulum and fills the whole nuclear cavity. Soon after the thread becomes retracted from the boundary and contracts towards one side of the cavity into a knot. ^(Fig 9) This stage persists for a sufficiently long time but the outline of thread remains perceptible. There is no sudden growth in the nucleus at this stage to give support to Lawson's view, that synizesis is caused by a sudden increase in the size of the nuclear cavity. The increase is steady and gradual. When the threads recover from synizesis contraction they extend more uniformly throughout the nucleus (Fig.10-12). This stage is known as open spirene stage. The opening and extension being very slow. The threads seem to be thicker than before. In some cases a second contraction is also reported but there is no such contraction in Momordica charantia.

There are seen definite small spherical deep staining bodies and are connected with the spirene at this stage.

HETEROTYPIC MITOSIS.

The threads break and are very long (Fig. 14). During a very early prophase these threads conjugate in pairs side by side parasynaptically. The association does not take place at all regions of the thread at once, it begins at one or two points, commonly at one end as described by Sharp (28), and gradually involves all portions, so that at stages when the process is yet incomplete, the two threads may be closely paired at some points and divergent at others. ^(Fig 15) After synizesis they continue to decrease in length and increase in diameter twisting round each other very tightly for a short time (Strapsinema, diplotene). ^(Fig 16) Eventually they become very short and thick, they are seen in pairs called bivalent chromosomes or gemini representing the reduced or the haploid number of chromosomes for the species. (Figs. 15 to 18).

They lie scattered in this condition through the nucleus (Diakinesis). The two components of each geminus now separate slightly at one point and represent chromosomes in the form of "X" and "O" (Fig. ¹⁷ 18). These bivalent chromosomes are now ready to take their places on the heterotypic mitotic spindle; each heterotypic chromosome now separates into two univalent chromosomes which gradually

move towards the poles and form two daughter nuclei bringing about reduction. The members of the bivalent chromosomes could be counted as eight definitely seen in diakinesis stage and the number of chromosomes (univalent) could be counted also as eight at the telophase plate and the metaphase plate of the first reduction division when cut across. No evanescent plate of the cell ^e at the equator of the spindle could be made out. In one case when the chromosomes have reached the poles one of them falls off (Fig. 20). There are also seen some black staining bodies on the spindle when the nuclei are going to be formed. (Fig. 21). While no such case has been referred to in any other plant worked out in the family.

HOMOTYPIC MITOSIS.

No wall is formed between the two daughter nuclei which again divide. The spindles of this division appear simultaneously and are arranged ~~at~~ neither at right angles to each other nor a parallel arrangement of the spindles has been seen, ^(Fig 25, 26) although Castetter(3) has referred to parallel spindles in Cucurbita maxima, and in Cucurbita pepo by Passmore(22). As soon as the four grand-daughter nuclei are formed at the poles of the two primary spindles these get connected as a rule with one another by systems of secondary achromatic fibrils in all possible directions which has not been seen in the preparation of Momordica charantia.

CYTOKINESIS.

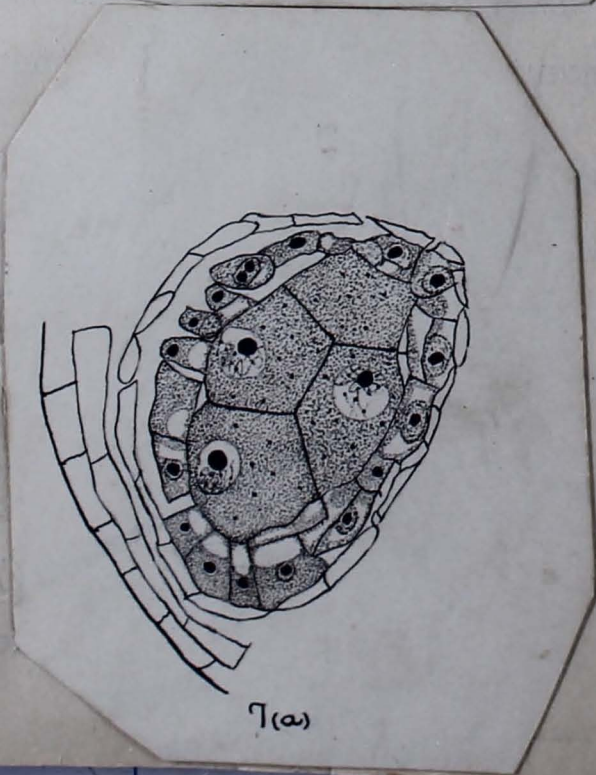
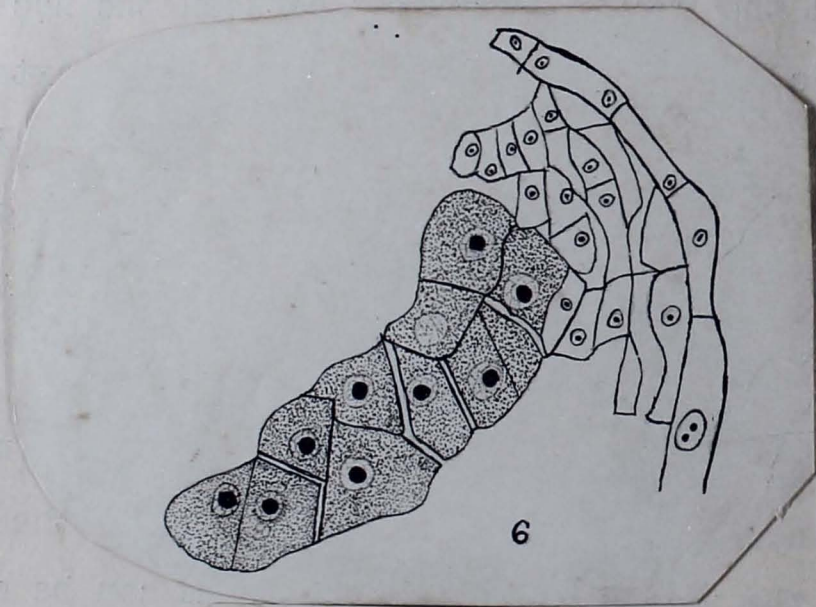
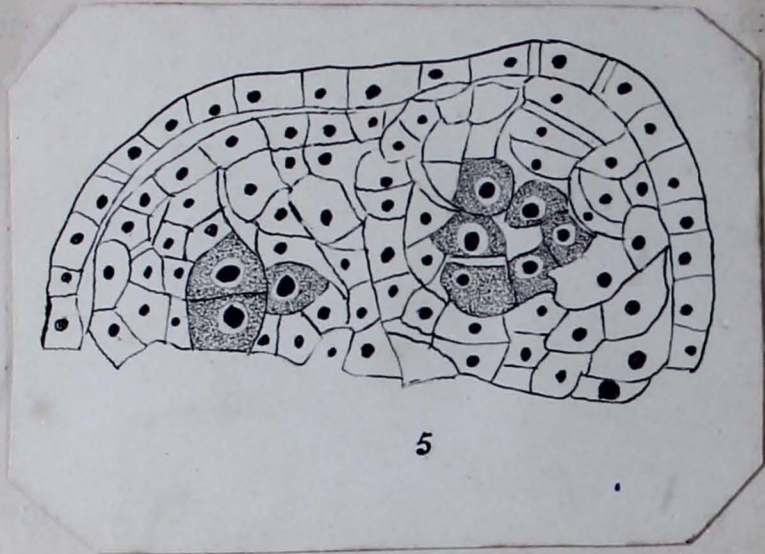
Castetter(3) stained the living material of Cucurbita maxima with Rosorcin blue showing that callose mother cell wall soon after heterotypic division had taken place, ^{was} distinctly laminated. Lamination being not evident in fixed material. He further adds "immediately surrounding the protoplast a new wall is now formed apparently by secretion from the mother cell wall. This special wall which is also composed of callose stains a deep blue and is more refractive than the outer wall. A similar thing was observed by him in Melilotus. In the case of Momordica charantia cytokinesis begins with a special wall probably the same callose wall referred by Castetter above, which immediately surrounds the cytoplasmic mass of the spore mother cell. After heterotypic division and homotypic division the daughter nuclei assume a tetrahedral arrangement lying as far apart from each other as possible. Spindles have already disappeared. There has been seen no sign of any vacuoles as reported by Castetter in Cucurbita maxima and Melilotus. Each side of the tetrahedron is practically an equilateral triangle with slightly convex faces and distinctly rounded corners (Fig. 32) There appear spindles between the nuclei (Fig. 32), clear but no so distinct as heterotypic and homotypic spindles are. The reason of their indistinct nature is given by Castetter(3) when he says "A careful examination of the preparation however shows that these spindle like regions are modifications in the form of cytoplasm and that the spindle like appearance is caused by the formation of the elongated vacuoles causing



the material on each side of each vacuole^{to} becomes stretched and therefore fibrous. That these are not real spindle fibres but normal elements of the cytoplasm is shown by their staining reactions, when Safranin and Gentian violet are used the chromatin^{and} cytoplasm stain red and pollen mother cell wall violet. If these regions extending between the nuclei were composed of two spindle fibres like those of heterotypic and homotypic spindles, the spindle fibres should stain violet. No change in the balance of the two stains was effected, however, in ~~the~~ securing a differential staining reaction between the peripheral region of the cytoplasm and the region extending between the nuclei". The writer concluded therefore that the regions extending between any two of the nuclei do not have true spindle fibres but are modifications in the form of the cytoplasm ". Kirkwood also shows this condition in Micrampelis at this stage, although he does not describe it.

In the case of Momordica charantia no doubt the spindles are not very distinct, still the entire absence of the vacuoles in the cytoplasm can be one and the only one argument to go against Castetter's result.

After this four conical projections appear on the inner surface of the so said special callose wall, These projections advance centripetally more rapidly than the connecting regions. Eventually the four ridges on the inner surface of the mother wall fuse at the centre of the cell cutting the mother cell into four uninucleate (some of them may be even bi or trinucleated) masses, the microspores (Figs. 27 to 34). Nagelis "Study of formation of micro-



spores in Cucurbita pepo describes cell plates for constriction, but he seems to have mistaken the ingrowing extension of the mother cell walls for cell plates because he was working on living material as criticised by Castetter(3). The walls which grow into the cytoplasm of the mother cells do not seem to be straight but are wavy as seen in(Fig.34)

After the septa have come together at the centre of the tetrad the special wall which surrounds each microspore becomes thicker and when the microspores are finally differentiated with an exine, the walls disintegrate; thus setting the microspores free in the cavity of the sporangium.

In one case the writer has seen three microspores disintegrated and they got a black stain while in others only one or two. So nothing can be said about the number of the degenerating microspores from each tetrad.

THE MICROSPORES

The separated microspores round up and enlarge(Fig.35) As usual a thick exine and a thin intine gets differentiated and the cytoplasm gets a little bit vacuolated. Vacuoles being not very clear. The exine is thick and stained very black with haematoxylin. It shows two to three lids which again stain very black. The shape of the lids is like a double convex lens. Coulter & Chamberlain(7) says "In certain cases a much more specialised method for the exit of pollen tube is provided, as among Cucurbitaceae and in

Passiflora, in which roundish lid like and often embossed pieces of the exine become detached". So the writer supports them in case of occurrence of lids, but they have not given any reference to the occurrence of germ pores in any other member of the family, while in the spores of Momordica charantia definite germ pores are to be seen which are from 2-3. The microspores may have both the lids and the germ pores at the same time. (Figs. 36 to 37). Both the lids and the germ pores may be present on the spore which shows a marked character and may be for sure fertilization, that is either the lids or the germ pores be used for the exit of the pollen tube.

Microspores have also got very faintly staining spines on the exine which are not present on the germ pores. The former view is also supported by Castetter(3) in Cucurbita maxima where he also gives the presence^{of} spines on the microspores. Sometimes very large vacuoles occur in the pollen grains. Nuclei are well marked.

GERMINATION OF THE MICROSPORE.

Begins with the division of its nucleus and occurs long before the dehiscence. Division results into a large tube nucleus and a small generative nucleus. Slowly they go apart. They have no distinct nucleoli present in them as are present in most of the plants as described by Coulter & Chamberlain(7). The generative nucleus reaching the peri-

phery, has got a definite separating wall formed. And therefore it resembles Typha, Sparganium, Naias, Convallaria, Neottia, Populus, Asclepias and Sarcodes described by Coulter & Chamberlain(7). Further division of the nucleus of the generative cell of the pollen grains inside the anther was seen to have taken place only α in a few cases. It forms two similar male nuclei which do not show special nucleoli as reported by Joshi(15), like most other Angiosperms. The nuclei are represented only by chromatin and no membrane but showing a definite cell of their own which does not stain as deeply as the rest of the microspor^e does. (Figs. 39-41)

The ripe pollen grains are particularly of the same size and shape, excepting in a few cases in which a little tube like bulging is observed on one side of it which is mostly in the shedding stage. Most of the large grains get degenerated, staining very black and having peculiar shapes. (Fig. 41a)

By this time the tapetum and all the wall layers of the anther except the outer epidermis and endothecium have disintegrated and have disappeared.

MEASUREMENTS.

The diameter of the protoplast in four stages of development was obtained by averaging the maximum and minimum diameters of five cells. The results are:-

Archesporium	Synizesis	First metaphase
14 μ	26 μ	28 μ .

Second telophase 28 μ

Microspore at tetrad stage 10 μ to 12 μ .

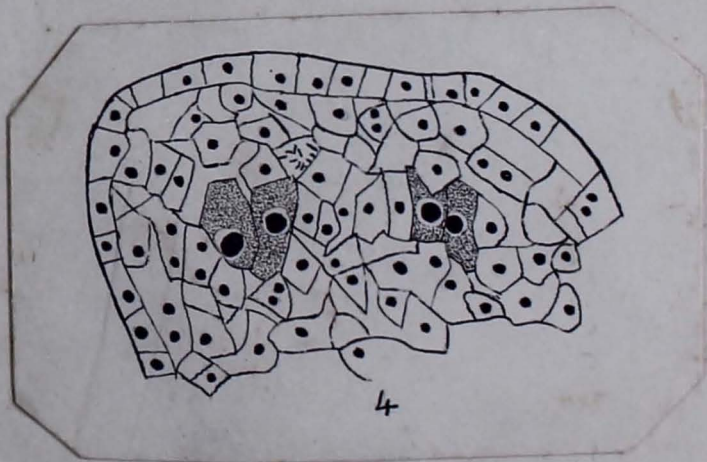
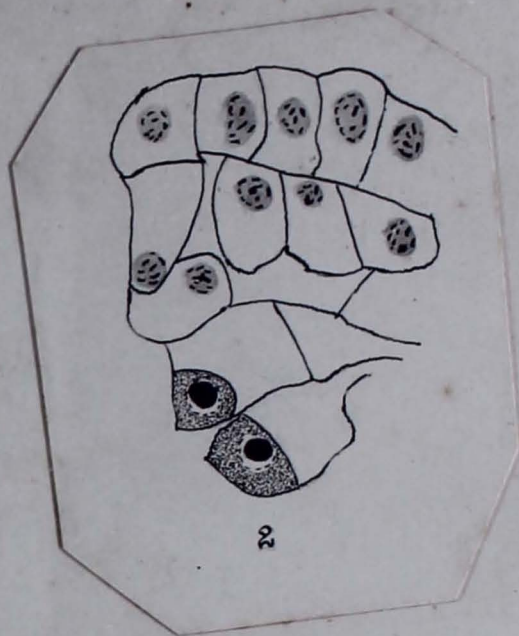
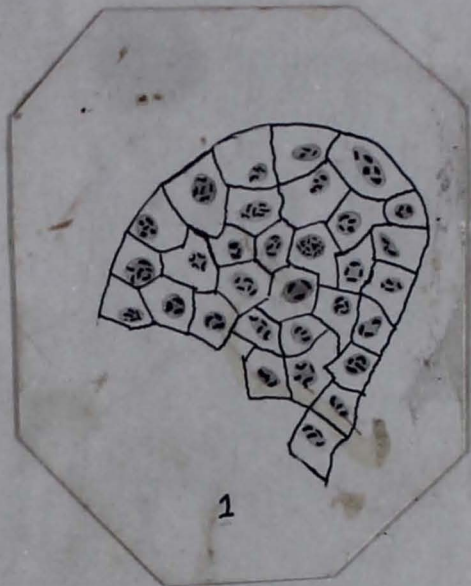
Microspore fully formed 42 μ to 47 μ .

This method of obtaining size of the cells is not strictly accurate but at least gives an idea of the great change in size that takes place as has been proposed by Passmore(22).

Momordica charantia

Plate I

Microsporogenesis



THE OVULE.

The microsporogenesis is at which stage when the archesporial tissue appears in the female flower cannot be said with certainty because the flowers are both male and female and not hermaphrodite. Ordinarily the microspore mother cells are in the process of reduction division when the archesporium first makes its appearance, the pollen grains are in a tetrad stage when the first division in the mega archesporial cell is over as occurs in Stelleria media (Joshi, 15).

Megasporogenesis.

Rudiments of an anatropous ovule arise on a fleshy bifurcating placenta which fills up the unoccupied space in the female ovary. At first each ovule consists of only a number of homogeneous meristematic cells, but later the cells seem to grow and multiply.

The primary archesporium differentiates very early before the integument is laid down. It may be hypodermal in origin because in Fig. 42, the anticlinally cut cells are very distinct which overlap the archesporial cell. It is not exactly hypodermal in origin ^{because} but the writer has seen in the preparations the

earliest stage to be a single cell occurring two layers below epidermis. The observations are in accordance with Gibs work on his species. The archesporium is recognised by the increasing size and different staining reaction of one of the cells and this single archesporial cell exactly goes in accordance with (Coulter & Chamberlain, 7) "Prevailing habit, however, is to limit the archesporium to the single hypodermal cell that terminates the axial row of the nucellus". This occurrence of a single celled archesporium is regarded by many people as an advanced character and many-celled archesporium as a primitive character, but many-celled archesporium also occurs in highly evolved and widely divergent families of Archichlamydeae like Umbelliferae and Compositae. Therefore it seems difficult to attach any systematic or phylogenetic importance to the presence or absence of a single-celled or many-celled archesporium, But a single-celled archesporium is a character with the Sympetalous Groups, (as is observed by the writer in Momordica charantia). Ordinarily, the archesporium gives rise by transverse divisions to a primary cell and a primary sporogenesis cell, but in the case of Momordica charantia there seems to be a complete suppression of parietal tissue, thus resembling monocot higher families, Commelina stricta of Commelinaceae (Guignard), Liliaceae (Allium, Hemerocallis, Lilium, etc.,) and many Archichlamydeae and Sympetalae in general. (Coulter & Chamberlain, 7) says, " The strongest

argument that suppression of the parietal tissue of the megasporangium is a strong tendency among Angiosperms is that this condition is universal among the Sympetalae". Therefore, the writer's observations also go in favour of the above said argument.

The archesporial cells now acts as a primary sporogenous cell itself or the spore mother cell as is given by Coulter & Chamberlain, 7" (but if all the sporogenous tissue is an archesporium, in this case contributed to by cells deeper than the hypodermal layer, the primary sporogenous cell does not divide, nor do all the archesporial cells give rise to a parietal cell. In any event the cells of the completed sporogenous mass whether archesporial or not are mother cells)". In longitudinal section of the nucellus of Momordica charantia in the beginning are only two to three anticlinally divided epidermal cells but these cells go on dividing more and more till the spore mother cell is forced very deep into the tissue of the nucellus as is clear from Figs. 43, 44, & 45, in the last case the megaspore mother cell is lying very deep.

The integument which is generally one in the case of Cucurbitaceae arises in a basipetal succession after the spore mother cell stage. It is composed of three to four layers of cells.

The megaspore mother cell now undergoes division to form the initial of the megaspore. The first division of this embryo-sac nucleus is a reduction division. This

is clear from the contraction of the spireme thread during the prophase and the reduced number of chromosomes at the two poles of the spindle of the early telophase (the exact number could not be counted but is definitely below 16). After the completion of the division one nucleus moves towards the chalazal end of the embryo-sac and the other towards the micropylar one. And the two nuclei form a wall between themselves and it seems as if there are two distinct megaspores.

After this as usual both these nuclei undergo a second division and that is to result in a row of four megaspores (Fig. 48). This corresponds to the tetrad stage in microsporogenesis. From the diagram and the inspection of the preparations it could not be concluded as to which of the four megaspores germinate further because all the four are on the same stage of development. There occurs a small space between the four megaspores as is clear from (Fig. 48) which may be due to defective fixation of the flowers. Most probably the innermost megaspore germinates further as is generally the case. There are definite vacuoles to be found in all the megaspores. The tetrad is not associated with a very small nucellus in this case while frequently in Sympetalae only the epidermal layer invests the tetrad row. Among Sympetalae there is found a row of three megaspores as well for example in Vaccinium and Lycium (Vesque), Lobelia (Marshall Ward) Lonicera and Nicotiana (Guignard) etc., but the writer's observations correspond with Sympetalae in general.

The lowermost functioning megaspore generally elongates and on germination encroaches upon the others until they become merely a cap upon it, or only show a darker region. Amon^g the Sympetalae there is an exception in Trapella in which Oliver finds the outermost of the four megaspores functions and in one case the next cell, while the innermost megaspore develops a remarkable haustorium. Under such diversity of opinion precluding as to which of the megaspore functions is very difficult.

In many preparations this stage is seen so that the four megaspore stage lasts for a sufficiently long time.

The argument in favour of the germination of the innermost megaspore is given by (Coulter & Chamberlain 7) as follows, " It should be noted in this connection that when a row of four megaspores is to be formed the nucleus near the chalaza almost invariably shows a more advanced stage in mitosis ^{at} ~~then~~ the nucleus nearest the micropyle, hence the megaspore at the chalazal end is formed a little earlier than the one at the micropylar end". A still more important reason for the selection of the chalazal megaspore is doubtless ^{its} ~~its~~ more immediate relation to the nutritive supply coming through the base of the ovule, a fact which may also count as another argument for the earlier mitosis at the chalazal end of the row.

From the megaspore stage onwards to the completion of the egg apparatus the history of the gametophyte is the same as is generally known.

The functional megaspore and its nucleus ~~is~~ usually enlarged very much before division, as is given by many authors. By the division the daughter nuclei migrate, one to each end of the sac (the stage has not been seen while inspecting the preparations). Subsequently divisions occur resulting in a four nucleate group at each end of the embryo sac.

During the process of the growth of embryo sac the surrounding nucellus tissue (whose most of the tissue is the result of many successive divisions of the epidermis because no parietal tissue is formed in this case), gets broken down staining a deep black.

The antipodal polar nucleus and the micropylar polar nucleus (sister to egg) then move towards one another and come to lie side by side in the general central region of the sac forming the primary endosperm nucleus (Fig. 49).²⁵⁰ The mature embryo sac of Momordica charantia is of the usual organisation consisting of an egg apparatus, three antipodals and two polar nuclei (Fig. 50). Egg apparatus at the micropylar end and consist of three cells (two synergids and one egg cell).

TRICHOSANTHES DIOICIA.

dioica

Trichosanthes dioecia.

It has got a very slender stem and is a climber. Tendrils are 2-4 fid. Leaves are generally three by two inches, ovate-oblong, chordate, acute, sinuate-dentate, not lobed, rigid and are rough on both surfaces. The petioles are $\frac{3}{4}$ inches, flowers are dioecious, male flowers are racemed, woolly outside. Calyx tube $1\frac{1}{4}$ inch, narrow; teeth linear, erect. Anthers are free. It is commonly found both wild and cultivated throughout the plains of North India extending to Assam and Eastern Bengal. Its fruit is used as a wholesome vegetable. Root, leaves and juice of the fruit are used medicinally.

Anther. Young stamen is bi-lobed from the very beginning when the meristematic cells begin to show their appearance. Meristem is covered over by an epidermis. As the anther grows the lobes go on becoming distinct. The primary archesporium could not be traced out and the earliest stage which the writer could find out was that of three-celled archesporium and two or three layers of parietal tissue in between the epidermis and the so said sporogenous tissue. The archesporial cells are differentiated by their usual features i.e. large in size possessing more densely staining cytoplasm and large nuclei. Outermost parietal layer becomes

differentiated as usual into an endothecium and the innermost layer into a tapetum. The tapetal cells are oblong shaped in section, broad large nuclei and richly protoplasmic with a capacity to stain more deeply than the microsporocytes. The greatest development of the tapetal cells in this case is during the tetrad stage. Here the tapetal cells are so much developed that the cells have divided three or four times to give rise to extraordinarily greater development. They definitely show a single nucleus in each cell throughout the life history of the plant as was observed in Momordica charantia. It seems that this condition of the tapetum is common in some of the Cucurbitaceae. There are definite vacuoles also present in the tapetal cells. The division of the sporogenous cells occur and as a result from twelve to twenty microspore mother cells are seen in the cross section of the anther lobe. In this case the microspore mother cells are much more smaller than those of the microspore mother cells of Momordica charantia and are definitely more in number than the above said plant., Therefore, naturally the pollen grain output of this plant will also be much more higher than the first plant. There are no darkly staining granules in the cytoplasm of the microspore mother cells as are seen in Momordica charantia.

Here also the mother cells were undergoing a period of rest do not round off but diverges from the

tapetal cells leaving in between faintly staining walls. The cytoplasm of the tapetum has also aggregated in one part of the cell.

The spore mother cells before entering into the first or the heterotypic reduction division are polygonal in outline. The cell walls being very thin. Each cell has one spherical nucleus in the centre with a fine nuclear membrane and no nucleolus present which should stain deeply than the rest of the nucleus as was the case in Momordica charantia showing thereby that these plants unlike most of the other Angiospermic plants are an exception. The stage of the appearance of the linen thread could not be found out but an advanced stage of the synzinesis was very clear as in (Fig. 54). No sudden increase in the size of the nuclear cavity could be found out. Here also synzinesis seems to persist for a sufficiently long time and the outline of the thread also remains perceptible. There is no second contraction seen in this plant as well. There are definite small spherical ~~spots~~ deep staining bodies present on the spireme. The doubling of the broken linen threads was very clear.

The heterotypic and homotypic spindles could not be found out, perhaps due to defective fixation and exhaustion which remained incomplete. But, the definite daughter nuclei formed after the completion of heterotypic and homotypic mitosis could be seen, e.g. as in (Figs. 59, 60 & 61).

No case was found in which the writer could count the chromosomes.

The Cytokinesis is also taking place by a definite so called callose wall as has been described in Momordica charantia. The tetrads are formed and there is no case in which the writer could see the number of degenerating microspores in a tetrad. Each cytoplasmic mass containing a single nucleus begins to round off and the spines which is a character of the family appear on the exine of the young pollen grain. At this stage the microspores are freed from the tetrad. The nucleus is small with a small membrane. After this the microspore begins to grow and a very thick exine is formed. The exine in this case is more thicker than that of Momordica charantia. The lids are also present on the exine as in (Fig. 66, 67). The writer could see in a few cases the presence of germ pores as well which supports the investigations of previous plant.

Usual differentiation in exine and intine occurs. There are present very big vacuoles in the advanced microspores. Here also there is a great variation in the size of the microspores.

The germination of the microspores results as usual in the formation of a bigger tube nucleus and a smaller generative nucleus. Generative nucleus when reaches the periphery forms a cell with a deep staining

protoplasm. There are no walls laid between the two cells and neither there is case of partition as was found in Momordica charantia. The writer could not find any case in which the generative nucleus has divided into the male nuclei. At the pollen shedding stage the tapetum and the wall layers of the anther and endothecium except the outer epidermis have disintegrated and disappeared.

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S U M M A R Y.

The primary archesporium in each sporangium of the anther consists of several cells. Microsporogenesis is almost typical with few exceptions so far not described,

- (a) Both lids and germ pores are present on the same microspore,
- (b) Presence of spines on the pollen grains,
- (c) Differentiation of generative cell,
- (d) Disorganisation of many pollen grains at the shedding stage,
- (e) Occurrence of darkly staining granules in spore mother cells of Momordica charantia and their complete absence in Trichosanthes dioica.

Mother cells do not round off and separate prior to division. Complete absence of nucleolus in open spireme stage is another exception to the rule. Tapetum is formed from parietal tissue and shows its highest development in Trichosanthes dioica at the tetrad stage where it is extraordinarily extensively divided. Again tapetum remains mononuclear throughout the life history of the plant, vacuoles are present in them. Cytokinesis takes place by furrowing supporting Castetter. The pollen output of each anther is from 600 to 800 in Momordica charantia while much more in Trichosanthes dioica. Pollen grains at the shedding stage are two to three nucleate.

The primary archesporium in ovule seems to be of a hypodermal origin. It does not divide to form a parietal tissue but the epidermis divides repeatedly to form a row of

cells and cover the so called spore mother cell which by ~~pressure~~ pressure is very deep seated. Only one integument. Tetrad is formed without any distinction in germinating and disintegrating cells. Embryo-sac is normal.

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EXPLANATION OF PLATES.Plate I.

- Fig.1. Showing meristematic cells of theca x580.
- Fig.2. Showing differentiation in the meristematic cells x1300.
- Fig.3. Sporogenous tissue cutting off parietal tissue x580.
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Plate II.

- Fig.5. Spore mother cells x580.
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- Fig.8. Spireme not yet clear, very faint, x900.
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 Fig.23. The same

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The megasporogenesis.

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